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PATENT

Attorney Docket No. A-64259-2/RMS/AMS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Not Assigned
NOLAN	Group Art Unit: Unknown
Serial No. 10/057,467	San Francisco, California
Filed: January 22, 2002	
For: METHODS FOR SCREENING FOR TRANSDOMINANT EFFECTOR PEPTIDES AND RNA MOLECULES	

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, Washington, DC 20231 on

Dated March 1, 2002

Signed Lisa Jeanetta
Lisa Jeanetta

SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

A Preliminary Amendment was filed in the above referenced case on February 13, 2002. A Petition to Correct Inventorship Pursuant to CFR 1.48(b) accompanied the Preliminary Amendment. Applicants respectfully wish to clarify by proper procedures that the inventorship is to be amended to delete S. Michael Rothenberg as inventor. Accordingly, please amend the above-referenced application as indicated below.

Please delete S. Michael Rothenberg as inventor.

Serial No.: 10/057,467
Filed: January 22, 2002

A copy of the pending claims are attached for the Examiner's convenience. Please direct any calls in connection with this application to the undersigned attorney. Although no fees are believed due, the Commissioner is authorized to charge any fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-64259-2/RMS/AMS).

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Anne M Shyjan

Anne M. Shyjan, Reg. No. 47,086, for
Robin M. Silva, Reg. No. 38,304

Dated: Mar. 1, 2002

Four Embarcadero Center, Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

1075958

Serial No.: 10/057,467
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PENDING CLAIMS:

8. A method for in vitro screening for a transdominant intracellular bioactive agent capable of altering the phenotype of a cell, said method comprising the steps:

a) introducing a molecular library of retroviral vectors comprising randomized candidate nucleic acids into a plurality of cells, wherein each of said nucleic acids comprises a different nucleotide sequence, wherein said randomized candidate nucleic acids are expressed in said cells to produce a plurality of randomized peptides, wherein each of said retroviral vectors comprises a nucleic acid encoding at least one glycine N-terminal to the randomized peptide;
b) screening said plurality of cells for a cell exhibiting an altered phenotype, wherein said altered phenotype is due to the presence of a transdominant bioactive agent; and
c) identifying said transdominant bioactive agent.

9. A method according to claim 8 wherein said identifying comprises:

i) isolating said cell exhibiting an altered phenotype.

10. A method according to claim 9 wherein said identifying further comprises:

ii) sequencing said nucleic acid encoding said transdominant bioactive agent.

11. A method according to claim 8 wherein each of said nucleic acids further comprise a presentation sequence capable of presenting said expression product in a conformationally restricted form.

12. A method according to claim 8 wherein said cells are mammalian cells.

13. A method according to claim 8 wherein said library comprises at least 10^4 different nucleic acids.

14. A method according to claim 8 wherein said library comprises at least 10^5 different nucleic acids.

15. A method according to claim 8 wherein said library comprises at least 10^6 different nucleic acids.

16. A method according to claim 8 wherein said library comprises at least 10^7 different nucleic acids.

17. A method according to claim 8 wherein said library comprises at least 10^8 different nucleic acids.

Serial No.: 10/057,467
Filed: January 22, 2002

18. A method according to claim 8 wherein said library comprises at least 10^9 different nucleic acids.

19. A method according to claim 8 wherein each of said candidate nucleic acids is linked to nucleic acid encoding at least one fusion partner.

20. A method according to claim 19 wherein said fusion partner comprises a nuclear localization signal sequence.

21. A method for in vitro screening for a transdominant intracellular bioactive agent capable of altering the phenotype of a cell, said method comprising the steps:
a) introducing a molecular library of retroviral vectors comprising randomized candidate nucleic acids into a plurality of cells, wherein each of said nucleic acids comprises a different nucleotide sequence, wherein said randomized candidate nucleic acids are expressed in said cells to produce a plurality of randomized peptides;
b) screening said plurality of cells for a cell exhibiting an altered phenotype, wherein said altered phenotype is due to the presence of a transdominant bioactive agent, wherein said altered phenotype is cell growth; and
c) identifying said transdominant bioactive agent.

22. A method for in vitro screening for a transdominant intracellular bioactive agent capable of altering the phenotype of a cell, said method comprising the steps:
a) introducing a molecular library of retroviral vectors comprising randomized candidate nucleic acids into a plurality of cells, wherein each of said nucleic acids comprises a different nucleotide sequence, wherein said randomized candidate nucleic acids are expressed in said cells to produce a plurality of randomized peptides;
b) screening said plurality of cells for a cell exhibiting an altered phenotype, wherein said altered phenotype is due to the presence of a transdominant bioactive agent, wherein said altered phenotype is cell death; and
c) identifying said transdominant bioactive agent.

23. A method for in vitro screening for a transdominant intracellular bioactive agent capable of altering the phenotype of a cell, said method comprising the steps:
a) introducing a molecular library of retroviral vectors comprising randomized candidate nucleic acids into a plurality of cells, wherein each of said nucleic acids comprises a different nucleotide sequence, wherein said randomized candidate nucleic acids are expressed in said cells to produce a plurality of randomized peptides;
b) screening said plurality of cells for a cell exhibiting an altered phenotype, wherein said altered phenotype is due to the presence of a transdominant bioactive

agent, wherein said altered phenotype is a change in expression of cellular differentiation markers; and
c) identifying said transdominant bioactive agent.

24. The method according to claim 23, wherein said cellular differentiation markers are characteristic of T-cell activation.

25. The method according to claim 23, wherein said cellular differentiation markers are characteristic of B-cell activation.

PATENT
Attorney Docket No.: A-64259-2/RMS/AMS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

NOLAN, GARRY P.

Serial No. 10/057,467

Filed: January 22, 2002

For: METHODS FOR SCREENING FOR TRANSDOMINANT EFFECTOR PEPTIDES AND RNA MOLECULES

Examiner: UNKNOWN

Group Art Unit: 1631

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231 on:

Dated: May 6, 2002

Signed: Lisa Jeanetta
Lisa Jeanetta

PRELIMINARY AMENDMENT RE SEQUENCE LISTING

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

Sir:

This Amendment is in response to the Notice to File Corrected Application Papers mailed March 13, 2002. A copy of the notice is enclosed. It is being filed withing two months from the date of said notice, making this a timely response. While no fee is believed to be due, the Commissioner is authorized to charge any additional fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-64259-2/RMS/AMS).

Please amend the application as follows to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures in adherence with rules 37 C.F.R. § 1.821-1.825:

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 3, line 22, with the following rewritten paragraph:

— Figure 2. Creation of a library of random peptides in a retrovirus DNA construct by primed DNA synthesis (SEQ ID NOS:10-14).—

Please replace paragraph beginning at page 4, line 9, with the following rewritten paragraph:

– The introduced nucleic acids and resultant expression products are randomized, meaning that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. The library may be fully random or biased, e.g., in nucleotide/residue frequency generally or per position. For example, a biased library may encode peptides for interactions with known classes of molecules, such as SH-3 domain proteins, as defined by peptides containing XXXPPXPXX (where X=randomized residues; SEQ ID NO:1). In other embodiments, the nucleotides or residues are randomized within a defined class, e.g., of hydrophobic amino acids, of purines, etc. In any event, where the ultimate expression product is a nucleic acid, at least 10, preferably at east 12, more preferably at least 15, most preferably at least 21 nucleotide positions need to be randomized; more if the randomization is less than perfect. Similarly, at least 5, preferably at least 6, more preferably at least 7 amino acid positions need to be randomized; again, more if the randomization is less than perfect.–

Please replace paragraph beginning at page 9, line 26, with the following rewritten paragraph:

– A scheme for generating a peptide library in the pBabe Puro vector is shown in Figure 2. Primers for PCR were synthesized, purified and deprotected according to standard protocols. Primer 1, complementary to polylinker sequences in the pBabe Puro retroviral construct, has the sequence 5' GCT TAG CAA GAT CTC TAC GGT GGA CCK NNK NNK NNK NNK NNK NNK NNK NNK NNK NNC CCC ACT CCC ATG GTC CTA CGT ACC ACC ACA CTG GG 3' (SEQ ID NO:2). N represents any of the four bases; K is limited to G or T. Primer 2 has the sequence 5' GCT TAG CAA GAT CTG TGT GTC AGT TAG GGT GTG G 3' (SEQ ID NO:3) and is complementary to sequences within the pUC18 origin of replication. PCR was carried out for 8 rounds using primer 1, primer 2, Babe Puro as template, and a mixture of Taq DNA Polymerase (Promega) and Deep Vent DNA Polymerase (New England Biolabs) in a ratio of 128 Taq: 1 Deep Vent as described in Barnes (1994) Proc. Natl. Acad. Sci. USA, 91, pp. 2216-222O. The amplified PCR product was purified, digested with restriction enzymes Bgl II and Not I (Promega), purified again and ligated with the corresponding Bam HI-Not I fragment of pBabe Puro. After transformation the resulting library contained ~$2x10^8$ clones, greater than 80% of which contained inserts. –

Please replace paragraph beginning at page 10, line 12, with the following rewritten paragraph:

— Oligonucleotides were synthesized and purified according to standard protocols. The "library" oligonucleotides have the sequence 5' CTG GAG AAC CAG GAC CAT GGG C $(NNK)_{10}$ GGG CCC CCT TAA ACC ATT AAA T 3' (SEQ ID

NO:4) or 5' CTG GAG AAC CAG GAC CAT GGG CNN KNN KNN KCC TCC CNN KCC TNN KNN KGG GCC CCC TTA AAC CAT TAA AT 3'(SEQ ID NO:5). A third oligonucleotide ("constant"), complementary to the 3' ends of the library oligonucleotides, has the sequence 5'TCA TGC ATC CAA TTT AAT GGT TTA AG 3'(SEQ ID NO:6). As shown in Fig. 2, each library oligonucleotide is annealed to the constant oligonucleotide, converted to double stranded DNA with Sequenase (United States Biochemical) or Klenow (Promega), digested with restriction enzyme Bst XI (New England Biolabs), and purified and ligated with the appropriate Bst XI-digested retroviral construct. Transformation efficiencies are ~ 2 x 10^8 clones per microgram of ligated DNA, greater than 90% of which contain an insert. A representative retrovirus is shown in Fig. 4; see also, retroviral vector with presentation construct nucleotide sequence (SEQ ID NO:7). —

Please delete the paragraph and subheading beginning at page 10, line 24 and continuing through page 13, line 7, inclusive.

Please replace paragraph beginning at page 13, line 25, with the following rewritten paragraph:

— In some embodiments of the invention, expression products are localized to, or preferentially concentrated in, different subcellular compartments within cells, e.g., by using appropriate addition of addressins to a peptide presentation construct, see, Figure 3. Addressins are available for a wide variety of subcellular locales including the nucleus, Golgi, mitochondria, plasma membranes, endoplasmic reticulum, secretory granules, secreted, cell surface (extracellular domain with

random), cell surface (intracellular domain random), etc. For example, many proteins whose functions require entry into the cell nucleus include nuclear localization signal (NLS) sequences: generally short, positively charged (basic) domains that serve to direct the entire protein in which they occur to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic NLS's such as that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val (SEQ ID NO:8)), Kalderon (1984), et al., Cell, 39:499-509, and double basic NLS's exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp (SEQ ID NO:9)), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that NLSs incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann, Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990.—

On page 16, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification and claims have been amended to include a Sequence Listing and proper reference to the sequences therein. Attached hereto is a marked-up version of the

changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a Request to Use Computer Readable Form of Sequence Listing From Another Applicaton and a paper copy of the sequence information from that prior application. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

DORSEY & WHITNEY LLP

Dated: May 6, 2002

Robin M. Silva

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

Robin M. Silva, Reg. No. 38,304
Filed under 37 C.F.R. Section 1.34(a)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 3, line 22, has been amended as follows:

— Figure 2. Creation of a library of random peptides in a retrovirus DNA construct by primed DNA synthesis (SEQ ID NOS:10-14).—

Paragraph beginning at page 4, line 9, has been amended as follows:

– The introduced nucleic acids and resultant expression products are randomized, meaning that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. The library may be fully random or biased, e.g., in nucleotide/residue frequency generally or per position. For example, a biased library may encode peptides for interactions with known classes of molecules, such as SH-3 domain proteins, as defined by peptides containing XXXPPXPXX (where X=randomized residues; SEQ ID NO:1). In other embodiments, the nucleotides or residues are randomized within a defined class, e.g., of hydrophobic amino acids, of purines, etc. In any event, where the ultimate expression product is a nucleic acid, at least 10, preferably at east 12, more preferably at least 15, most preferably at least 21 nucleotide positions need to be randomized; more if the randomization is less than perfect. Similarly, at least 5, preferably at least 6, more preferably at least 7 amino acid positions need to be randomized; again, more if the randomization is less than perfect.–

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CNN KCC TNN KNN KGG GCC CCC TTA AAC CAT TAA AT 3'(SEQ ID NO:5). A third oligonucleotide ("constant"), complementary to the 3' ends of the library oligonucleotides, has the sequence 5'TCA TGC ATC CAA TTT AAT GGT TTA AG 3'(SEQ ID NO:6). As shown in ~~Fig. 3~~ Fig. 2, each library oligonucleotide is annealed to the constant oligonucleotide, converted to double stranded DNA with Sequenase (United States Biochemical) or Klenow (Promega), digested with restriction enzyme Bst XI (New England Biolabs), and purified and ligated with the appropriate Bst XI-digested retroviral construct. Transformation efficiencies are ~ 2 x 10^8 clones per microgram of ligated DNA, greater than 90% of which contain an insert. A representative retrovirus is shown in Fig. 4; see also, retroviral ~~nucleotide sequence below:~~ vector with presentation construct nucleotide sequence (SEQ ID NO:7). —

Paragraph beginning at page 10, line 24, has been deleted as follows:

– ~~Retroviral vector with presentation construct.~~

~~TGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTT~~

~~GCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGG~~

~~TTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGT~~

~~AAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGC~~

~~GGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGC~~

~~C~~

~~CCAAGGACCTGAAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTT~~

~~CGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAG~~

~~CCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTCGCCC~~

~~GGGTACCCGTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGG~~

~~ACTCGCTGATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCCACCTCG~~

~~GGGGTCTTTCATTTGGAGGTTCCACCGAGATTTGGAGACCCCTGCCTAGGG~~

~~ACCACCGACCCCCCCGCCGGGAGGTAAGCTGGCCAGCGGTCGTTTCCTGT~~

~~CTGTCTCTGTCTTTGTGCGTGTTTGTGCCGGCATCTAATGTTTGCGCCTGCG~~

~~TCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAAC~~

~~TGACGAGTTCTGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACT~~

~~TTGGGGGCCGTTTTTGTGGCCCGACCTGAGGAAGGGAGTCGATGTGGAAT~~

~~CCGACCCCGTCAGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACA~~

~~GTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGAACCGAAGCCGCGC~~

~~GTCTTGTCTGCTGCAGCGCTGCAGCATCGTTCTGTGTTCTCTCTGTCTGACT~~

~~GTGTTTCTGTATTTGTCTGAAAATTAGGGCCAGACTGTTACCACTCCCTTA~~

~~AGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAACCA~~

~~GTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAAT~~

~~GGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAGAC~~

~~CTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACAC~~

~~CCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCC~~

~~CCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCT~~

~~CCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGA~~

~~TCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCGGAATTCCAGGA~~

~~CCATGGGCGGGCCCCCTTAAACCATTAAATTGGTAAAATAAAGGATCCGT~~

~~CGACCTGCAGCCAAGCTTATCGATAAAATAAAAGATTTTATTTAGTCTCCA~~

~~GAAAAAGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTT~~

~~AAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGA~~

~~AGTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAAC~~

~~AGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGA~~

~~TGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGA~~

~~TGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCCTTATTTGAA~~

~~CTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGA~~

~~GCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGAT~~

~~AGACTGCGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGC~~

~~ATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTG~~

~~ACTACCCGTCAGCGGGGGTCTTTCATTCGTAATCATGGTCATAGCTGTTTC~~

~~CTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGA~~

~~AGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATT~~

~~AATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA~~

~~GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTG~~

~~GGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC~~

~~TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACA~~

~~GAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA~~

~~AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTC~~

~~CGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG~~

~~AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC~~

~~TCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCT~~

~~TTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATC~~

~~TCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCC~~

~~CCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA~~

~~ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG~~

~~ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG~~

~~GCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT~~

~~GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAC~~

~~AAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGC~~

~~GCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCT~~

~~GACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATT~~

~~ATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAA~~

~~ATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTT~~

~~AATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGT~~

~~TGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCAT~~

~~CTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCA~~

~~GATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG~~

~~GTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAG~~

~~CTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTG~~

~~CTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCT~~

~~CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAA~~

~~AAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCC~~

~~GCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTC~~

~~ATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCA~~

~~TTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATA~~

~~CGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG~~

~~AAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGAT~~

~~CCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTA~~

~~CTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA~~

~~AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCT~~

~~TTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATA~~

~~CATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACAT~~

~~TTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACA~~

~~TTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTC~~

~~GGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCAC~~

~~AGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGT~~

~~CAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAG~~

~~CAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATG~~

~~CGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCA~~

~~ACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG~~

~~GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTT~~

~~TTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCACGCTCTCCCTTAT~~

~~GCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGC~~

~~ACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTC~~

~~CCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATG AGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATA GGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTC CGGCGTAGAG~~ –

Paragraph beginning at page 13, line 25, has been amended as follows:

— In some embodiments of the invention, expression products are localized to, or preferentially concentrated in, different subcellular compartments within cells, e.g., by using appropriate addition of addressins to a peptide presentation construct, see, Figure 3. Addressins are available for a wide variety of subcellular locales including the nucleus, Golgi, mitochondria, plasma membranes, endoplasmic reticulum, secretory granules, secreted, cell surface (extracellular domain with random), cell surface (intracellular domain random), etc. For example, many proteins whose functions require entry into the cell nucleus include nuclear localization signal (NLS) sequences: generally short, positively charged (basic) domains that serve to direct the entire protein in which they occur to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic NLS's such as that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val (SEQ ID NO:8)), Kalderon (1984), et al., Cell, 39:499-509, and double basic NLS's exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp (SEQ ID NO:9)), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that

NLSs incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann, Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990.—

On page 16, immediately preceding the claims, the enclosed "SEQUENCE LISTING" was inserted into the specification.